Superantigen Vaccines: A Comparative Study of Genetically Attenuated Receptor-Binding Mutants of Staphylococcal Enterotoxin A

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Superantigens exert their pathologic effects by direct binding to major histocompatibility complex (MHC) class II molecules and T cell antigen receptors (TCR), thus circumventing the normal, antigen-specific immune response. A direct link between disease and toxin suggests an excellent opportunity for vaccine intervention. Site-directed mutants of staphylococcal enterotoxin A (SEA) that have attenuated binding to either the TCR or the MHC class II molecule were developed. Both kinds of SEA mutants induced high levels of antibody against SEA when used as vaccines, and the immunized animals were fully protected when challenged with wild type toxin. However, a residual lethality was associated with the attenuated TCR-binding mutant. These results, combined with an understanding of the molecular nature of superantigen and receptor interactions, indicate that targeting MHC class II binding by site-directed mutagenesis will produce the most effective vaccine.

Bacterial superantigens are ligands for both major histocompatibility complex (MHC) class II molecules, expressed on antigen-presenting cells, and the variable portion of the T cell antigen receptor β chain (TCR Vβ) [1–4]. Each bacterial superantigen has a distinct affinity to a set of TCR Vβ, and coligation of the MHC class II molecule polyclonally stimulates T cells [5–7]. Pathologically elevated levels of cytokines that are produced by activated T cells are the probable cause of toxic shock symptoms [8, 9]. In addition, susceptibility to lethal gram-negative endotoxin shock is enhanced by several bacterial superantigens [9].

Staphylococcal enterotoxins (SEs) A through E are the most common cause of food poisoning [10] and are associated with several serious diseases [11, 12], such as bacterial arthritis [13, 14], other autoimmune disorders [15], and toxic shock syndrome [16, 17]. The nonenterotoxic staphylococcal superantigen toxic shock syndrome toxin-1 was first identified as a causative agent of menstrual-associated toxic shock syndrome [18]. Superantigen-producing *Staphylococcus aureus* strains are also linked to Kawasaki syndrome, an inflammatory disease of children [19]. Although antibodies reactive with superantigen are present at low levels in human sera [20], boosting antibody titers by specific immunization may be efficacious for patients at risk for toxic shock syndrome and the other disorders of common etiology.

A vaccine approach to controlling bacterial superantigen–associated diseases presents a unique set of challenges. Acute exposure to bacterial superantigens produces T cell anergy [21], yet T cell help is presumably a requirement for mounting an antibody response. The superantigen vaccines used in this study were developed by engineering changes in the receptor-binding portions of SEA to reduce receptor-binding affinities and toxicity while maintaining antigenicity.

A three-dimensional structural homology model of SEA complexed with the MHC class II molecule was developed [22] from the structural alignment of SEA and unbound SEB molecules [23] and cocrystallographic data of the complex between SEB and the human MHC class II molecule, HLA-DR1 [24]. MHC class II molecule and TCR-binding modes, confirmed by site-specific mutagenesis, are conserved between SEB and SEA [22]. The MHC class II–binding surface of SEA and SEB consists of a conserved polar pocket and hydrophobic loop. Mutation of a critical tyrosine (SEA residue 92) within the polar pocket is postulated to disrupt hydrogen bonding to lysine 39 of the DRα subunit and results in a 1000-fold reduction in SEA binding affinity. TCR interactions map to nonconserved residues within a shallow surface cavity of SEA [12] and SEB [25]. Thus, the mutation tyrosine 64 to alanine, within this site of SEA, results in diminished T cell recognition [12].

Immunization strategies based on attenuation of either TCR or MHC class II receptor binding were compared. We determined whether antibody responses to both types of engineered vaccines protected immunized mice from SEA challenge.

Materials and Methods

SEA mutants. The production of SEA wild type (WT) or mutant genes and protein expression in *Escherichia coli* were done as described [22]. In brief, bacteria were grown to an A_{600} of 0.5–0.6 in Terrific Broth (Difco, Detroit) containing 50 μg/mL ampicillin. Bacteria were collected by centrifugation, washed with

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In conducting the research described herein, the authors adhered to the Guide for Laboratory Animal Facilities and Care as promulgated by the Committee on the Guide for Laboratory Animal Resource/NRC.

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30 m*M* NaCl and 10 m*M* TRIS (pH 7.6), and pelleted by centrifugation. Periplasmic proteins were recovered from the bacterial pellet as described [22] and concentrated (Centriprep10; Amicon, Beverly, MA).

The SEA preparations were partially purified by CM Sepharose (Sigma, St. Louis) chromatography, using a linear gradient of 10–50 m*M* potassium phosphate (pH 5.8–7.4). The partially purified SEA was further purified by preparative isoelectric focusing (MinipHor; Rainin Instrument, Woburn, MA). The MinipHor was loaded with the SEA-enriched fraction from CM Sepharose chromatography in a solution containing 10% (vol/vol) glycerol and 1% (vol/vol) ampholytes, pH 6–8 (Protein Technologies, Tucson). The protein preparations were allowed to focus until equilibrium was reached (~4 h, 4°C). Twenty focused fractions were collected, and aliquots of each were analyzed by SDS-PAGE and immunoblotting (as described below). The SEA-containing fractions were pooled and refocused for an additional 4 h. The fractions containing purified SEA were pooled and dialyzed first against 1 *M* NaCl (48 h, 4°C) to remove ampholytes and then against buffer consisting of 50 m*M* sodium phosphate and 140 m*M* sodium chloride, pH 7.4 (PBS), for 12 h at 4°C.

The concentrations of recombinant proteins were determined by the bicinchoninic acid method (Pierce, Rockford, IL) and quantitative immunoblotting, using an affinity-purified anti-SEA antibody and the WT SEA as a standard protein, as described below. All protein preparations were >99% pure, as judged by SDS-PAGE and Western immunoblotting.

Gel electrophoresis and immunoblotting analysis. The SEA preparations were analyzed by SDS-PAGE (12%) and stained with Coomassie brilliant blue R-250 (Sigma) in methanol (10% vol/vol) acidic acid (10% vol/vol). The proteins separated by SDS-PAGE (not stained) were transferred to nitrocellulose membranes (Bio-Rad, Melville, NY) by electroblotting, and the membranes were then blocked (12 h, 4°C) with 0.2% casein in PBS. The membrane was then incubated (1 h, 37°C, shaking) with 2 μg/mL affinity-purified anti-SEA antibody (Toxin Technology, Sarasota, FL) in PBS with 0.02% casein. After the membranes were thoroughly washed, peroxidase-conjugated goat anti-rabbit IgG (Cappel/Organon Teknika, West Chester, PA) was added (1:5000) and the membranes were incubated for 1 h (37°C) with shaking. The unbound antibody was removed by washing with PBS, and bound antibody was visualized by using a Bio-Rad peroxidase development kit. For quantitation, dilutions of SEA preparations were immobilized on nitrocellulose membranes by using a slot-blot apparatus (Bio-Rad). The membrane was removed from the slot-blot apparatus, and unreacted sites were blocked (12 h, 4°C) with 0.2% casein in PBS. After washing once with the PBS, the membrane was incubated (1 h, 37°C) with 2 μg/mL rabbit affinity-purified anti-SEA antibody (Toxin Technology) in PBS that contained 0.02% casein. After four washes, the bound rabbit antibody was reacted with goat anti-rabbit IgG conjugated with horseradish peroxidase (1 h, 37°C), and the blots were developed using enhanced chemiluminescence (Amersham Life Sciences, Arlington Heights, IL). The amount of WT SEA or mutant protein was measured by densitometry (NIH Image 1.47 software) of exposed radiographic film. Standard curves were prepared by plotting the mean of duplicate densitometric readings for each dilution of WT SEA standard. The resulting values were fitted to a straight line by linear regression. Concentrations of SEA mutant proteins were determined by comparing mean values of various dilutions of the mutant to the standard curve.

Biologic activities and immunizations. Male C57BL/6 mice, 10–12 weeks old, were obtained from Harlan Sprague-Dawley (Frederick Cancer Research and Development Center, Frederick, MD). The lethal effect of WT or mutant SEA was evaluated as described for WT SEA [9]. For immunizations, mice were given by intraperitoneal injection either 2 or 10 μg of WT or mutant SEA protein in 100 μL of RIBI adjuvant (RIBI Immunochem Research, Hamilton, MT), or adjuvant only, and boosted intraperitoneally at 2 and 4 weeks. Serum was collected from tail veins 1 week after the last immunization. Mice were challenged 2 weeks after the last injection with WT SEA (10 μg) and lipopolysaccharide (LPS, 150 μg) from *E. coli* O55:B5 (Difco) as described [9]. Challenge controls were adjuvant-immunized or nonimmunized mice injected with both agents (100% lethality) or with either SEA or LPS. No lethality was produced by these negative controls.

Antibody assay. Microtiter plates were coated with 1 μg/well WT SEA in 100 μL of PBS (37°C, 2 h). After antigen coating, the wells were blocked with 250 μL of casein 0.2% in PBS for 4 h at 37°C and then washed four times with PBS containing 0.2% Tween 20. Immune or nonimmune mouse sera were diluted in PBS containing 0.02% casein, and 100 μL of each dilution was added to duplicate wells. After each well was washed four times, bound antibody was detected with horseradish peroxidase (Sigma)–labeled goat anti-mouse IgG (37°C, 1 h), using *o*-phenylenediamine as the chromogen. The mean of duplicate optical densities (absorbance at 490 nm) of each treatment group was determined, and these data were compared on the basis of the inverse of the highest serum dilution that produced an optical density reading four times above the negative control wells. For negative controls, antigen or serum was omitted from the wells.

T lymphocyte proliferation assay. Splenic mononuclear cells were obtained as described [9]. Briefly, mononuclear cells were isolated by buoyant density centrifugation (Sigma) and washed three times. The cells were resuspended in medium containing 5% fetal bovine serum, and 100 μL (4×10^5 cells) of the cell suspension was added to triplicate wells of 96-well flat-bottom plates. The mononuclear cells were cultured (37°C, 5% CO_2) with WT or mutant SEA. After 3 days the cultures were pulsed (12 h) with 1 μCi/well [^{3}H]thymidine (Amersham), and incorporated radioactivity was measured by liquid scintillation.

SEA binding and TCR subset analysis. Cells from the mouse B lymphoma line A20 ($2–4 \times 10^5$ cells) were incubated (40 min at 37°C) with WT or mutant SEA in Hanks' balanced salt solution containing 0.5% bovine serum albumin (HBSS). The cells were washed with HBSS and incubated with 5 μg of affinity-purified anti-SEA antibody in HBSS (4°C, 45 min). Unbound antibody was removed, and the bound antibody was detected with fluorescein isothiocyanate–labeled goat anti-rabbit IgG (Organon Teknika). Unbound antibody was removed and the cells were analyzed by a FACSort flow cytometer (Becton Dickinson, Mountain View, CA).

For TCR subset analysis, splenic mononuclear cells were obtained from mice immunized with WT or mutant SEA. The mononuclear cells were incubated (37°C) with WT SEA (100 ng/mL) for 5 days and then cultured in 85% RPMI 1640 and 10% interleukin-2 supplement (Advanced Biotechnologies, Columbia, MD) with 5% fetal bovine serum for an additional 5 days. The T cells were

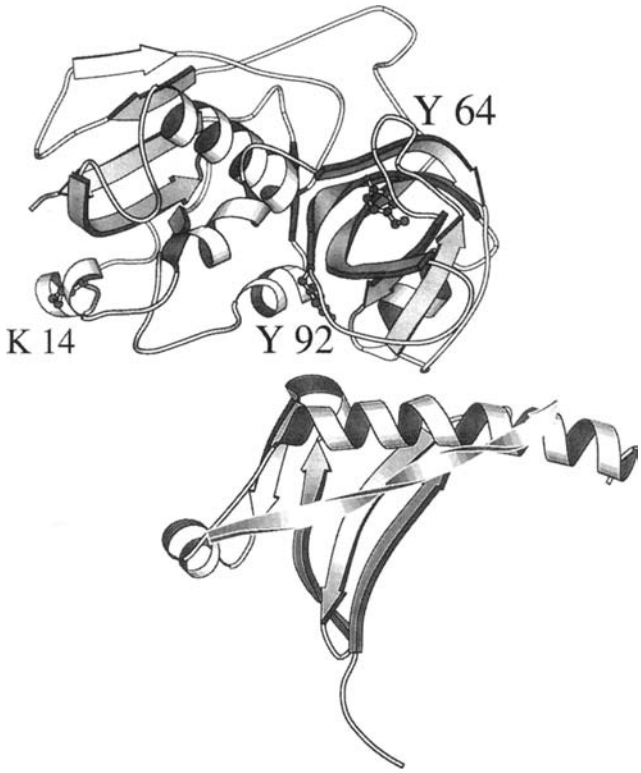

Figure 1. Positions of amino acid residues that have been mutated in staphylococcal enterotoxin A (SEA) vaccines. Residue K14E is located in N-terminal peptide of SEA that does not interact with either major histocompatibility complex (MHC) class II molecule or T cell receptor (TCR). Residue Y64A is involved in TCR interactions, while residue Y92A is critical for MHC class II binding [22].

washed twice and stained with anti-TCR (Biosource, Camarillo, CA) or anti-Vβ–specific TCR (45 min, 4°C). All cells analyzed were positive for the T cell marker CD3 and expressed the CD25 activation marker (data not shown). Controls were incubated with an isotype-matched antibody of irrelevant specificity. Unreacted antibody was removed, and the cells were incubated with a fluorescein isothiocyanate–labeled anti-mouse IgG (Organon Teknika) on ice for 30 min. The cells were washed and analyzed by flow cytometry (FACSort).

Results

SEA mutants. The predicted structure of SEA in complex with the human MHC class II molecule HLA-DR1 is shown in figure 1. For clarity, only the DRα features most critical to SEA binding and the location of the mutated SEA amino acids are shown. Substituting SEA tyrosine 92 with alanine (Y92A) reduces DR1 binding 1000-fold [22]. Tyrosine 64 is located in a site that interacts with TCRs, and mutation to alanine (Y64A) substantially reduced human T cell responses [22]. Lysine 14 is located at the N-terminus of SEA in a region exhibiting weak secondary structure and does not participate in interactions with the MHC class II molecule or TCR [22]. We therefore mutated lysine 14 to glutamic acid (K14E) and used this SEA mutant as a control.

After purification, a single homogenous protein band, migrating just under 30 kDa, was detected for WT or mutant SEA by SDS-PAGE (figure 2A). A faint second 60-kDa band, corresponding to the previously reported molecular dimer of SEA [26], was observed with Western blots probed with anti-SEA antibody (figure 2B).

TCR and MHC class II binding of engineered SEA mutants. The binding of WT or mutant SEA was evaluated with the MHC class II–expressing murine B cell lymphoma cell line A20 (figure 3). The binding affinity of WT SEA to mouse MHC class II (H-2^d) molecules was lower than that observed with human MHC class II–expressing cells (data not shown), reflecting the reduced toxicity that bacterial superantigens exert in mice [9]. WT SEA, Y64A, and K14E all had the same relative affinity to mouse MHC class II molecules. Similar to the results obtained with human MHC class II molecules [22], the Y92A, when compared with WT SEA, mutant exhibited substantially reduced binding to A20 cells (figure 3).

The effect of WT SEA or site-specific SEA mutants on splenic mononuclear cells obtained from nonimmunized C57BL/6 (H-2^b) mice is shown in figure 4. Both WT SEA and the control mutant K14E were potent T cell activators, effective at minimal concentrations of 10–100 pg/mL. However, T cell responses to Y92A were reduced at least 100-fold compared with responses to WT SEA, while Y64A-stimulated responses were slightly higher than those to Y92A. These results confirmed that attenuation of superantigen binding to either MHC class II or TCR molecules resulted in dramatically reduced mouse T cell proliferation.

To investigate the physiologic effects of the attenuated SEA mutants, toxicities were examined in vivo (table 1). Mice are ordinarily refractory to the lethal effects of bacterial superantigens. However, when mice are injected with nonlethal levels

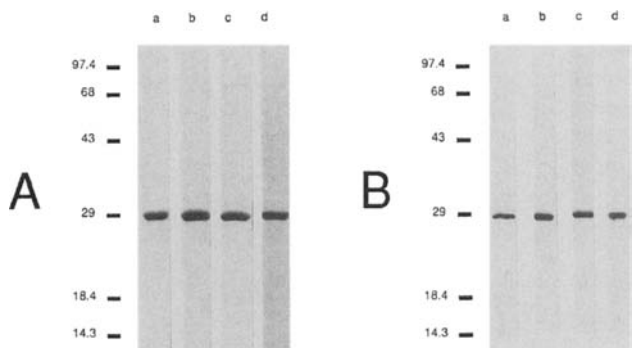

Figure 2. SDS-PAGE (**A**) and Western immunoblot (**B**) analysis of purified staphylococcal enterotoxin A (SEA) wild type (WT) and mutant proteins. WT or mutant SEA were boiled in SDS (1%) and 2-mercaptoethanol (5%) and separated on 2 identical gels (12% polyacrylamide). Proteins were stained with Coomassie blue. Lanes a–d correspond to WT, K14E, Y64A, and Y92A, respectively.

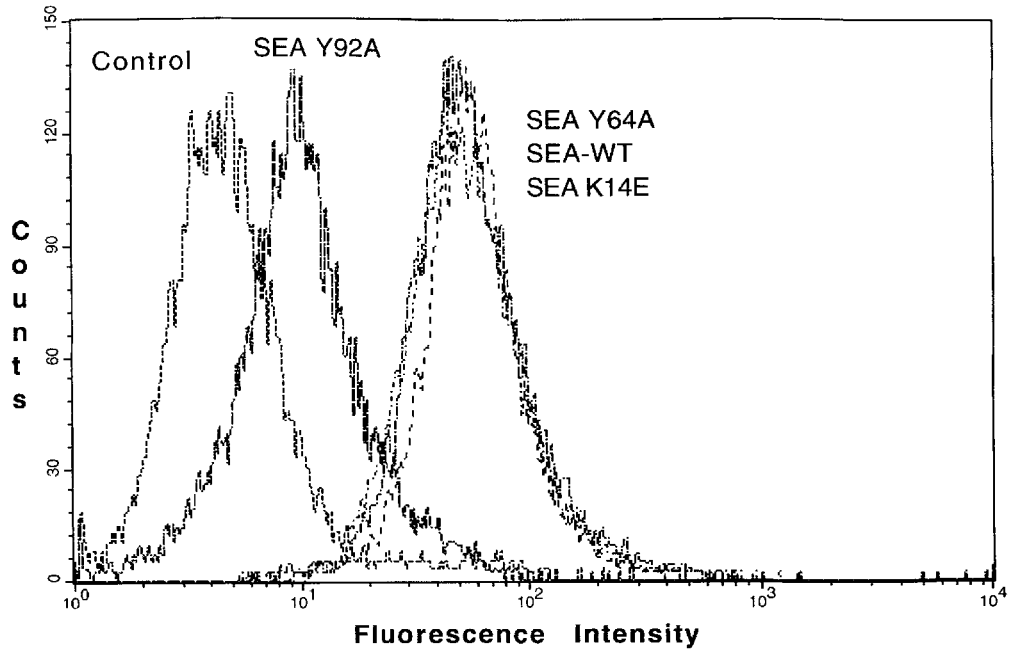

Figure 3. Staphylococcal enterotoxin A (SEA) mutant Y92A has attenuated binding to major histocompatibility complex class II molecules. Mouse B cell lymphoma cells, A20, were incubated with 1 μg of wild type (WT) or mutant SEA. Unbound SEA was removed, and cells were incubated with affinity-purified anti-SEA antibody. Bound antibody was detected by flow cytometry using fluorescein isothiocyanate (FITC)–labeled anti-rabbit IgG. Negative control consisted of A20 cells reacted sequentially with primary and FITC-labeled antibodies without addition of SEA.

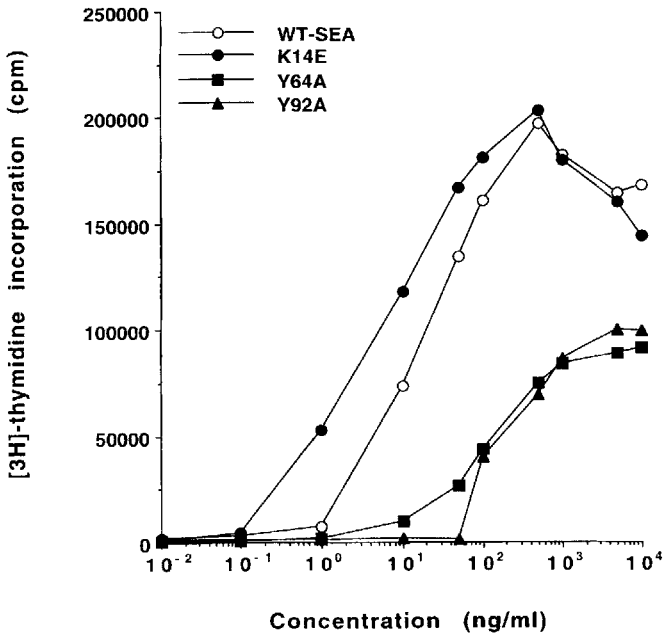

Figure 4. Mutants of staphylococcal enterotoxin A (SEA) that have either attenuated T cell receptor or major histocompatibility complex class II binding are very weak superantigens. Results are mean counts/min of lymphocyte cultures incubated with wild type (WT) or mutant SEA for 72 h and then pulse-labeled for 12 h with [^{3}H]thymidine. SDs of triplicate wells were ≤10%. $P = .0001$ (analysis of variance for repeated measures comparing Y64A and Y92A to WT SEA at concentrations of 10–10,000 ng/mL).

of LPS from gram-negative bacteria, a fatal toxic shock results that is characterized by elevated levels of proinflammatory cytokines such as tumor necrosis factor-α, interleukin-6, and interferon-γ [9]. The SEA concentration that was selected for this experiment represents 10 LD_{50} [9]. WT SEA, site-specific SEA mutants (10 μg/mouse each), or LPS (150 μg/mice) injected alone were nonlethal to mice. However, combining LPS with either WT SEA or mutant K14E resulted in 100% lethality. For those mice receiving both LPS and WT or K14E SEA, 80% were dead by 24 h and 100% by 48 h. In contrast, 100% of Y92A- and 80% of Y64A-injected mice (coadministered with LPS) survived. The average time to death for the 20% of mice that did not survive Y64A injection was 48–72 h. These in vivo data correlated well with the results obtained with the lymphocyte cultures. It was concluded that the observed attenuation of toxicity in mice was a direct result of the reduced T cell proliferation.

Superantigen vaccine. Having established that attenuation of receptor binding resulted in reduced toxicity, we next examined the immunogenicity of the SEA mutants. Mice were im-

Table 1. Biologic effect of wild type (WT) staphylococcal enterotoxin A (SEA) and SEA mutants.

Protein	No. live/total
WT	0/10
K14E	0/10
Y64A	8/10
Y92A	10/10

NOTE. Mice were given 10 LD_{50} (10 μg) of WT or mutant SEA. Lipopolysaccharide (150 μg/mouse) was injected 3 h later.

Table 2. Mice immunized with attenuated forms of staphylococcal enterotoxin A (SEA) produce high titers of neutralizing antibody.

Immunizing agent	Dose (μg/mouse)	Anti-SEA (WT) antibody titer*	No. live/total
WT	2	10,000–50,000	10/10
	10	10,000–50,000	10/10
K14E	2	5000–10,000	8/10
	10	10,000–50,000	10/10
Y64A	2	5000–10,000	6/10
	10	10,000–50,000	10/10
Y92A	2	1000–5000	2/10
	10	10,000–50,000	10/10
Adjuvant		50–100	0/10

NOTE. Mice were given 10 LD_{50} of wild type (WT) SEA challenge followed by potentiating dose of lipopolysaccharide (150 μg/mouse) 3 h later.

* Reciprocal of serum dilution resulting in optical density reading four times above negative controls (wells containing either no SEA or no primary antibody).

munized with WT or mutant SEA. Control mice received adjuvant only or were left untreated. One week before challenge with WT SEA, blood samples were taken from mice, and serum antibody titers were determined for each group (table 2). Mice immunized with 2 μg of Y64A or Y92A had serum antibody titers of 1:5000 and 1:1000, respectively. Immunization with 2 μg of WT SEA or control mutant resulted in titers of 1:5000 and 1:10,000, respectively. The highest immunizing dose (10 μg/mouse) was most effective for all animals, resulting in antibody titers >1:10,000. All mice were challenged with 10 LD_{50} of WT SEA (potentiated with LPS). The survival data correlated well with the levels of serum antibodies in immunized mice. All mice that were vaccinated with 10 μg of Y64A or Y92A survived the lethal challenge dose of WT SEA. Slightly less protection was afforded by the lower vaccination dose of mutant Y64A or Y92A. All mice immunized with both doses of WT SEA survived the lethal challenge with WT SEA. Mice immunized with mutant K14E exhibited survivals of 100% and 80% for high and low vaccination doses, respectively. All nonimmunized or control mice that were vaccinated with adjuvant alone died when challenged with WT SEA and a potentiating dose of LPS.

Immune recognition of superantigen mutants. Bacterial superantigens induce clonal anergy of specific subsets of T cells in mice. It was possible that the loss of sensitivity to WT SEA among the mice vaccinated with the attenuated mutant forms represented a state of specific nonresponsiveness instead of specific immunity. To address this issue, lymphocyte responses to WT SEA were measured with splenic mononuclear cells collected 2 weeks after the third immunization (figure 5). As expected, lymphocytes from mice that were immunized with WT SEA or control SEA mutant showed little to no proliferation when incubated with the WT superantigen. In contrast, lymphocytes obtained from control mice or those immunized with either Y64A or Y92A all responded vigorously to the WT SEA (figure 5). The TCRs used by T cells from the SEA-vaccinated mice were then characterized by flow cytometry. T cells from immunized or control mice were incubated with WT SEA in culture for 7 days, followed by a 5-day expansion in interleukin-2–containing medium. Distinct populations of activated TCR Vβ11–positive cells were observed with T cells from mice immunized with Y92A and Y64A (figure 6), representing 48% and 40% of T cells, respectively. However, Vβ11-expressing cells obtained from WT SEA– or K14E-immunized mice were ~1% and 6% of the total T cell population, respectively, suggesting that this subset was nonresponsive to restimulation with the WT superantigen. T cells bearing Vβ17a, 3, 7, and 10b were unchanged for all mice (data not shown). It was apparent that T cell responses to both the TCR and MHC class II binding–attenuated SEA mutants were similar to each other but differed from responses to control or WT molecules. These results suggested conventional antigen-processing was

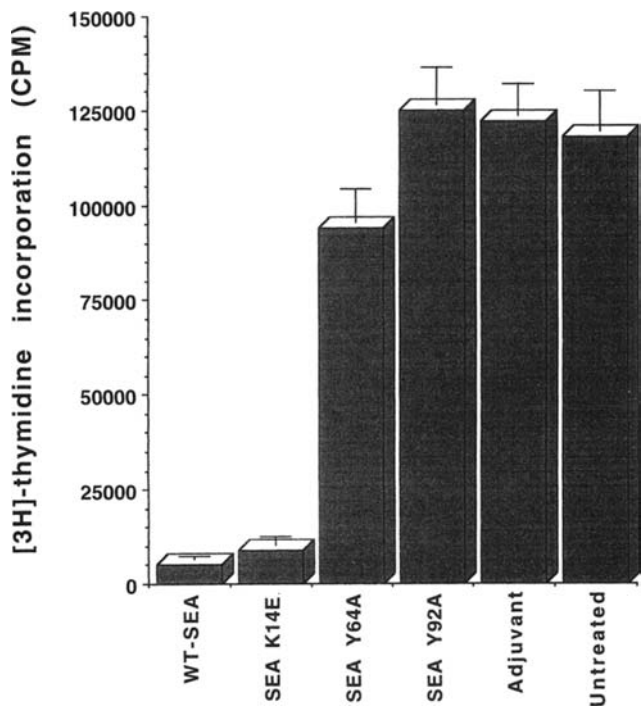

Figure 5. Mutant staphylococcal enterotoxin A (SEA) vaccines that have attenuated major histocompatibility complex class II or T cell receptor binding do not induce T cell anergy. Mice were given three doses of wild type (WT) SEA or site-specific mutant vaccine, plus adjuvant. Control animals received adjuvant alone or were untreated; 2 weeks after final injection, pooled mononuclear cells were collected from spleens of 4 mice from each group. Results are represented as mean cpm (±SD) of quadruplicate wells incubated with 100 ng/mL WT SEA for 72 h and then pulse-labeled for 12 h with [^{3}H]thymidine. $P < .0001$ (analysis of variance for repeated measures comparing untreated, adjuvant, Y64A, and Y92A to WT SEA group).

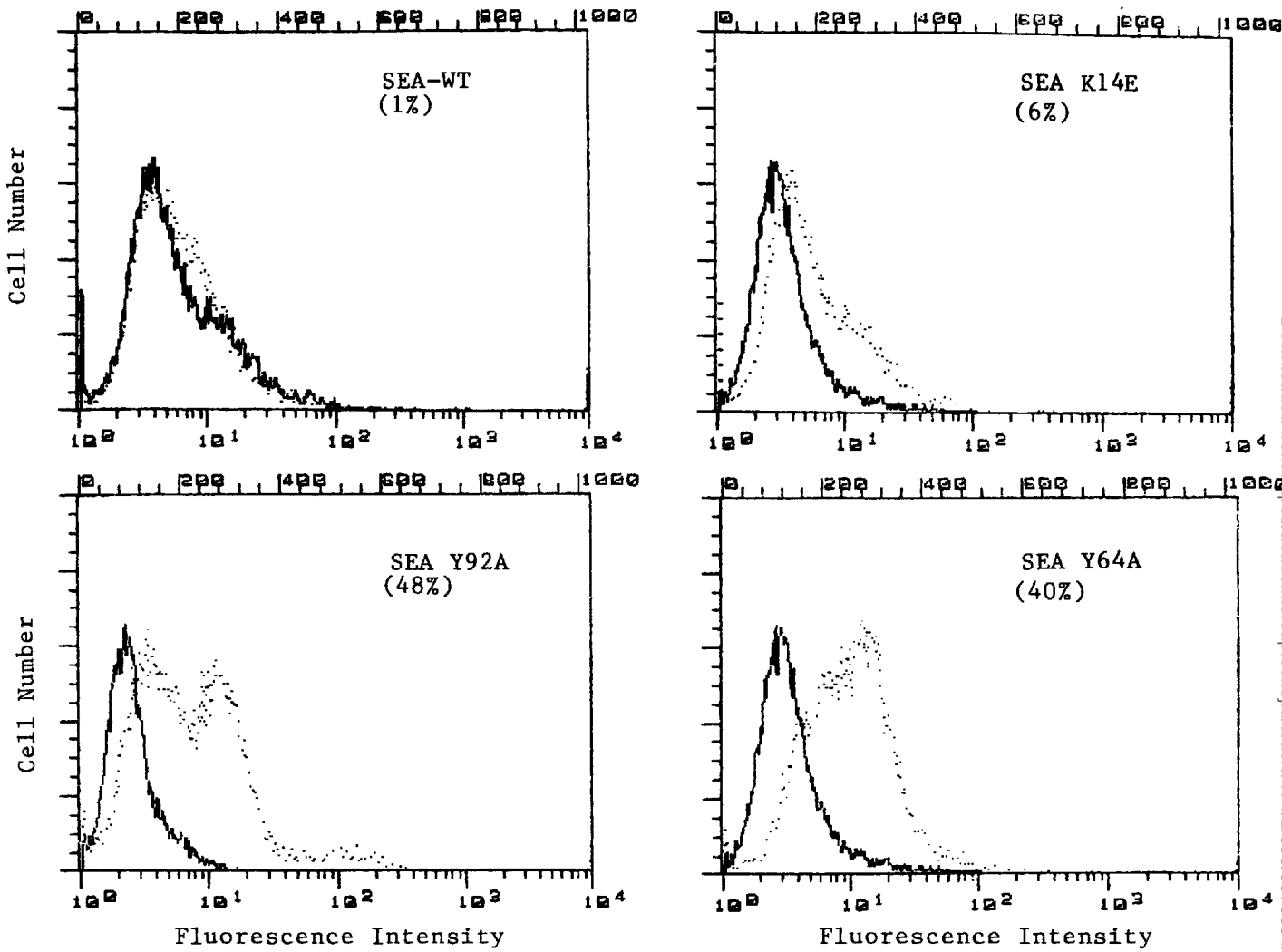

Figure 6. Mice immunized with attenuated staphylococcal enterotoxin A (SEA) mutant vaccines do not exhibit selective depletion of T cells bearing T cell receptor Vβ11 phenotype. T cells from vaccinated mice were probed for expression of Vβ11 (dotted lines) using flow cytometry and compared to control antibody (solid line). Percentages of T cells expressing Vβ11 are shown in parentheses. Abscissa = log fluorescence, ordinate = no. of events (cells). WT, wild type.

functioning in presentation of the superantigen mutants Y64A and Y92A.

Discussion

Binding of SEA and other superantigens to HLA-DR molecules is characterized by a fast on-rate and high affinity [27]. All defined bacterial superantigens interact with MHC class II molecules by means of a common DRα subunit binding mode, with additional stabilizing interactions that are unique to each [12]. A second, zinc-dependent, DRβ subunit binding mode for SEA, SED, and SEE [28] increases MHC class II binding but is not directly involved with T cell signaling. Both the Y92A and Y64A mutants used in the present study were expected to bind to the DRβ subunit because the residues required to form the zinc coordination complex have not been altered. The leucine at position 48 of SEA is located in a solvent-exposed reverse-turn on the surface of the molecule (the hydrophobic binding loop), and this structural motif is conserved among all bacterial superantigens [12]. The hydrophobic binding loop and the residues forming the polar binding pocket are likely to be the most important structural features driving strong interactions with the DRα subunit [22].

Antigen presentation molecules of the MHC play a critical role in controlling immune responses by selectively activating T cells that express receptors complimentary to bound peptides. In contrast, superantigens activate T cells with little apparent regard for antigen specificity. For SEA, the dominant TCR subsets that are activated include Vβ1, 3, 10, 11, 12, and 17 [7]. Direct ligation of T cell antigen receptors, independent of

MHC molecules, may occur in some cases [29]. However, by targeting MHC class II molecules, a more effective subterfuge of the immune response occurs. Peptides presented by MHC class II molecules in some cases are sterically blocked by bound bacterial superantigens [30, 31], thus circumventing the normal immune recognition of antigens. The responding lymphocytes are subsequently removed from their role as effector cells. Most T cells that are activated by superantigen are eliminated by a Fas/Fas ligand–dependent apoptosis [32], or they may enter into a state of specific nonresponsiveness. However, as our data demonstrated, when the strong MHC class II receptor interactions were attenuated by site-directed mutagenesis, the superantigen was effectively recognized by the immune system and an appropriate antibody response ensued. Although T cell anergy was not apparent when the SEA mutant Y64A was used as an immunogen, a residual amount of toxicity was observed compared with the results obtained with the MHC class II–binding mutant Y92A. Because TCR-binding modes of superantigens are not conserved [22], mutating key amino acid residues in these sites may actually result in the acquisition of a new TCR recognition repertoire [25]. Therefore, our results indicate that attenuation of MHC class II binding provides the best route for vaccine development.

The first critical step in SEA activation of T cells is binding of the superantigen to the MHC class II molecule. Antibody inhibition of this interaction should be sufficient to prevent initiation of the disease sequelae. Based on structural homologies, it is conceivable that antibody responses against one superantigen will protect against other bacterial superantigens. If this proves to be correct, then a multivalent vaccine made up of a minimal combination of superantigen subunits, with attenuated MHC class II binding, may be effective against the majority of superantigens. However, the protein surfaces that are predicted to interact with TCRs are not conserved [22], suggesting that a vaccine strategy targeting T cell interactions is likely to be effective only for a limited number of superantigens.

Mice immunized with the attenuated SEA mutant Y92A were fully protected from challenge with the WT toxin, and high levels of antibody against WT SEA were detected in the sera of all mice receiving the vaccine. Therefore, we conclude that immunization with the nonsuperantigenic form of SEA can elicit a strong neutralizing antibody response. In addition, Y64A and Y92A did not induce T cell anergy. T cell responses of both WT and control SEA–vaccinated mice were depleted of Vβ11 T cells, while this population of lymphocytes was highly responsive in mice immunized with the mutants Y64A or Y92A. This observation indicates that anergy induction in vivo requires engagement of both TCR and MHC class II molecule. These data also suggested that these two mutants were potent immunogens because conventional processing and presenting to T cells had occurred.

It is known that primates are most sensitive to SEA and related bacterial superantigens; therefore, it will be necessary to also address toxicity and immunogenicity issues directly in these species. Finally, we anticipate that the availability of a potential superantigen vaccine will allow a direct assessment of the putative role of these bacterial products in a number of human diseases.

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